Part of #10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Richard Kroczek

Serial No.: 09/509,283	Group Art Unit: 1644
Filed: August 11, 2000	Examiner: J. Roark
For: ANTI-HUMAN T-CELL COSTIMULATING POLYPEPTIDE MONOCLONAL ANTIBODIES (AS AMENDED)	Attorney Docket No.: 7853-215 (new docket no.)

DECLARATION OF RICHARD KROCZEK UNDER 37 C.F.R. § 1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Richard Kroczek, declare that:

1. I am the inventor of the invention described and claimed in the above-identified patent application (the "'283 application").

2. I presently hold the position of Professor of Molecular Immunology at the Robert Koch Institute, Berlin, Germany, the assignee of the above-identified patent application.

3. The invention claimed in the '283 application is directed to monoclonal antibodies directed against a human polypeptide, referred to in the '283 application as the "8F4 polypeptide," hybridoma cells that produce the monoclonal antibodies, and pharmaceutical compositions that comprise the monoclonal antibodies. Since the filing date of the '283 application, the 8F4 polypeptide has come to be referred to in the literature as "ICOS" (Inducible T cell CO-Stimulator). A polypeptide corresponding to an 8F4 polypeptide has also been referred to in the literature as "H4."

4. I conceived of and reduced to practice the claimed subject matter of the '283 application prior to November 1996.

5. The conception and reduction to practice of the invention claimed in the '283 application is evidenced by Exhibits 1-4, annexed hereto. The dates of Exhibits 1-4 have been redacted in accordance with standard practice, but all are prior to November 1996. The experiments described and summarized in the annexed Exhibits 1-4 were done by me or under my direction and supervision. The experiments were carried out in Germany, a World Trade Organization (WTO) member country, prior to November 1996.

6. Anti-8F4 monoclonal antibodies were produced as part of a project to generate monoclonal antibodies directed against activated T cell antigens, in particular 2-signal-activated T lymphocyte antigens. The monoclonal antibodies were generated by Dr. Barbara Eljaschewitsch, a junior physician in my laboratory. Dr. Eljaschewitsch was an inexperienced worker whose project and experiments were performed under my direction and supervision. The hybridoma fusion described herein that expresses the anti-8F4 monoclonal antibody described in the annexed exhibits was generated with the assistance of Ulf Korthäuer, who at the time was a doctoral student in my laboratory. The experiments contributed to by Mr. Korthäuer were performed under my direction and supervision.

7. Briefly, the strategy for generating monoclonal antibodies directed against 2-signal-activated T lymphocyte antigens involved: (a) initial immunization and subsequent boosting of mice with purified human T cells that had been activated with a 2-signal stimulus (*i.e.*, PMA and A23187, which are pharmacological agents used for *in vitro* 2-signal T cell stimulation); (b) fusion of spleen cells of the immunized mice with myeloma cells to produce myeloma-spleen fusion hybridomas; and (c) identification of a monoclonal antibody produced by a hybridoma that is directed against a 2-signal activated T lymphocyte antigen.

8. A laboratory sheet summarizing the hybridoma fusion experiment designated "Ba-1," which resulted in the generation of 8F4 monoclonal antibody, is annexed hereto as Exhibit 1. The exhibit presents the laboratory sheet in three forms: a copy of the handwritten original, a German transcript of the original, and an English translation of the transcript. As discussed below, a hybridoma of the Ba-1 fusion experiment, designated "8F4," recognizes an antigen that is expressed on 2-signal-activated T lymphocytes but is absent from non-activated T lymphocytes when the cells are stained with an 8F4 monoclonal antibody coupled to the fluorochrome FITC. The 8F4 hybridoma produces the 8F4 monoclonal antibody described and utilized in the examples presented in the '283 application. The antigen produced on 2-signal-activated T lymphocytes is the 8F4 polypeptide described throughout the '283 application.

9. The experimental details of the T-cell preparation and 2-signal cell activation procedures, and of the immunization of mice summarized in Exhibit 1 are provided in the page taken from one of Mr. Korthäuer's laboratory notebooks, a copy of which page is annexed hereto as Exhibit 2. The exhibit presents the laboratory notebook page in three forms: a copy of the handwritten original, a German transcript of the original notebook page, and an English translation of the transcript.

10. One of the hybridomas of the Ba-1 fusion experiment, the 8F4 hybridoma, satisfied the screening criteria by recognizing a molecule on 2-signal-activated T cells. Exhibit 3, annexed hereto, presents the results of fluorescence activated cell sorting (FACS) flow cytometry experiments demonstrating that the monoclonal antibody produced by the 8F4 hybridoma ("mAb 8F4") recognizes an antigen (the 8F4 polypeptide) present on the surface of T lymphocytes 2-signal-activated for 40 hours. All the flow cytometry experiments described herein were performed on a Coulter EPICS machine. Printouts generated by the machine are given a date and a continuous, unique number, both of which are fixed and cannot be changed by the experimenter.

11. Exhibit 4, annexed hereto, presents the results of additional FACS flow cytometry experiments. These results demonstrate: a) that the mAb 8F4 monoclonal antibody does not recognize an antigen present on non-stimulated T lymphocytes (4-1); and b) that the mAb 8F4 monoclonal antibody recognizes the 8F4 antigen present on the surface of T lymphocytes 2-signal-activated for 12 hours. As above, the the flow cytometry experiments described herein were performed on a Coulter EPICS machine. Printouts generated by the machine are given a date and a continuous, unique number, both of which are fixed and cannot be changed by the experimenter.

12. In summary, as evidenced by the attached Exhibits 1-4, which represent experiments performed in Germany, a WTO member country, by me or under my supervision and direction, I conceived of and reduced to practice the anti-8F4 monoclonal antibody-related subject matter claimed in the '283 application prior to November 1996.

13. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of any patent issuing from this application.

Respectfully submitted,

Date May 21, 2001

Richard Kroczek

Attachments:

Exhibit 1: Copy of myeloma-spleen fusion experiment data sheet describing the Ba-1 fusion experiment. Three forms of the data sheet are included: a copy of the handwritten original, a German transcript of the original, and an English translation of the transcript.

Exhibit 2: Copy of a page taken from one of Mr. Korthäuer's laboratory notebooks. Three forms of this page are included: a copy of the handwritten original, a German transcript of the notebook page, and an English translation of the transcript.

Exhibit 3: mAb 8F4 flow cytometry experiment results demonstrating the recognition of the 8F4 polypeptide by mAb8F4 on human T cells 2-signal-activated for 40 hours.

Exhibit 4: mAb 8F4 flow cytometry experiment results demonstrating that mAb 8F4 does not recognize an antigen on non-stimulated human T cells (4-1), and does recognize the 8F4 polypeptide on human T cells 2-signal-activated for 12 hours (4-2).